ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

Gene	Disease (MONDO ID)	Clinically significant transcript
ITGA2B	**Glanzmann thrombasthenia (MONDO: 0010119)**	**NM_000419.4**
ITGB3	**Glanzmann thrombasthenia (MONDO: 0010119)**	**NM_000212.2**

Pathogenic Criteria		
Criteria	**Original Criteria Description**	**Specification(s)**
Very Strong Criteria		
PVS1	Null variant in a gene where LOF is a known mechanism of disease	Use decision tree as per SVI WG with specified "regions critical to protein function".
PS2/PM6_VeryStrong	*De novo* in a patient with disease and no family history	Use proposed SVI point recommendations. - Only applicable when proband has a known pathogenic or likely pathogenic variant with the *de novo* variant.
PM3_VeryStrong	For recessive disorders, detected in trans with a pathogenic variant	Use proposed SVI point recommendations. Both variants must be classified using *ITGA2B/ITGB3* Rule Specifications.
Strong Criteria		
PS1	Same amino acid change as a previously established pathogenic	Use with no specification.

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

	variant regardless of nucleotide change	
PS2/PM6_Strong	*De novo* in a patient with disease and no family history	Use proposed SVI point recommendations. - Only applicable when proband has a known pathogenic or likely pathogenic variant with the *de novo* variant
PS3	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	- In a transgenic animal model, must demonstrate minimal to no function. -OR- - In a model organism or heterologous cell line, EITHER (A) when expression is normal or reduced, disruption of protein function must be demonstrated OR (B) Absent surface protein expression (<5%).
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Rule does not apply due to rarity of disorder and lack of appropriate studies.
PM3_Strong	For recessive disorders, detected in trans with a pathogenic variant	Use proposed SVI point recommendations. Both variants must be classified using *ITGA2B/ITGB3* Rule Specifications.
PP1_Strong	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Segregations in proband plus >2 affected relatives. *Affected relatives must have both variants identified in proband.
PP4_Strong	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Proband with clinical diagnosis of GT based on the presence of mucocutaneous bleeding and appropriate lab abnormalities. Full sequencing of both genes is required at this strength.

Moderate Criteria		
PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation	Rule does not apply due to genes being highly polymorphic.
PM3	For recessive disorders, detected in trans with a pathogenic variant	Use proposed SVI point recommendations. Both variants must be classified using *ITGA2B/ITGB3* Rule Specifications.
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	Use with no specification.
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Use with no specification.
PS2_Moderate/PM6	*De novo* in a patient with disease and no family history	Use proposed SVI point recommendations. - Only applicable when proband has a known pathogenic or likely pathogenic variant with the *de novo* variant

PS3_Moderate	Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product	In a model organism or heterologous cell line, significantly reduced surface protein expression (5-25%).
PP1_Moderate	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Segregation in proband plus 2 affected relatives. *Affected relatives must have both variants identified in proband.
PP4_Moderate	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Proband with clinical diagnosis of GT based on the presence of mucocutaneous bleeding and appropriate lab abnormalities.
Supporting Criteria		
PM2_Supporting	Absent in population databases (or at extremely low frequency if recessive)	Prevalence <1/10,000 (<0.0001) alleles in gnomAD.
PP1	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Segregation in proband plus 1 affected relative. *Affected relatives must have both variants identified in proband.
PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease	This rule does not apply because benign missense variants are not rare.
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	REVEL score of $\geq$ 0.7 -OR-

		>2 independent *in silico* missense predictors predict a damaging impact
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.	As per SVI recommendation, do not use this rule.
PS2/PM6_Supporitng	*De novo* in a patient with disease and no family history	Use proposed SVI point recommendations. - Only applicable when proband has a known pathogenic or likely pathogenic variant with the *de novo* variant
PM3_Supporting	For recessive disorders, detected in trans with a pathogenic variant	Use proposed SVI point recommendations. Both variants must be classified using *ITGA2B/ITGB3* Rule Specifications.
PM5_Supporting	Novel missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before	Use at adjusted strength.

Benign Criteria

Criteria	Original Criteria Description	Specification(s)
Stand Alone Criteria		
BA1	Allele frequency is greater than expected for disorder	Frequency cutoff of 0.24% (>0.0024 at 99.99% CI w/subpopulation w/min of 5 alleles).

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

Strong Criteria		
BS1	Allele frequency is greater than expected for the disorder	Frequency cutoff of 0.158% (>0.00158 at 99.99% CI w/subpopulation w/min of 5 alleles)
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	>1 homozygote who is unaffected proven with at least aggregometry.
BS3	Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	- Must demonstrate normal aggregometry in a transgenic mouse model. -OR- - In a heterologous cell line, must demonstrate BOTH normal expression and normal protein function.
BS4	Lack of segregation in affected members of a family	Variant not detected in an affected family member.
Supporting Criteria		
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	Rule does not apply as truncating variants do not predominate and missense variants are a known cause of disease.
BP2	Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed *in cis* with a pathogenic variant in any inheritance pattern.	Use as written for recessive variants (i.e. - variant must be observed *in cis* with a pathogenic variant).
BP3	In-frame deletions/insertions in a repetitive region without a known function	Use with no specification.
BP4	Multiple lines of computational evidence suggest no impact on gene/gene product	REVEL score of < 0.25

Related publication(s):

Date Approved: September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

BP5	Variant found in a case with an alternate molecular basis for disease	Do not use this rule as an individual can be a carrier of an unrelated pathogenic variant for a recessive disorder.
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	As per SVI recommendation, do not use this rule.
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	Use with no specification.

RULES FOR COMBINING PATHOGENIC CRITERIA – Unchanged at this time

PATHOGENIC

1. 1 Very Strong AND
    a. ≥1 Strong OR
    b. ≥2 Moderate OR
    c. 1 Moderate and 1 Supporting OR
    d. ≥2 Supporting
2. ≥2 Strong OR
3. 1 Strong AND
    a. ≥3 Moderate OR
    b. 2 Moderate AND ≥2 Supporting OR
    c. 1 Moderate AND ≥4 Supporting

LIKELY PATHOGENIC

1. 1 Very Strong AND 1 Moderate OR
2. 1 Strong AND 1-2 Moderate OR
3. 1 Strong AND ≥2 Supporting OR
4. ≥3 Moderate OR
5. 2 Moderate AND ≥2 Supporting OR
6. 1 Moderate AND ≥4 Supporting
7. 1 Very strong AND 1 Supporting

RULES FOR COMBINING BENIGN CRITERIA - Unchanged at this time

Related publication(s): **Date Approved:** September 4, 2020

Benign

1. 1 Stand-Alone OR
2. ≥2 Strong

Likely Benign

1. 1 Strong and 1 Supporting OR
2. ≥2 Supporting

Introduction to Glanzmann thrombasthenia (GT)

Glanzmann thrombasthenia (GT) is an inherited platelet disorder, in which platelets fail to aggregate to physiologic stimuli due to quantitative or qualitative defects in integrins αIIb or β3 (GPIIb/IIIa). Of note, the classical/standard definition of GT was not based on genotype, and is primarily a phenotypic diagnosis based on clinical presentation and platelet aggregation, and does not consider protein defects other than integrins αIIb or β3.

Clinical phenotype: moderate-severe mucocutaneous bleeding; platelets fail to aggregate to physiologic stimuli, but agglutinate to ristocetin; vast majority with normal platelet number and size. Historically, patients grouped as Type I (<5% αIIbβ3) type II (5-15% [or 5-25%] αIIbβ3) and type III or variant GT with normal levels of dysfunctional αIIbβ3.

Inheritance: primarily autosomal recessive; compound heterozygotes predominate outside ethnic groups.

Genetic epidemiology/Incidence: GT is a rare disease (defined in U.S. as less than 1 in 200,000). Some sources estimate an incidence of 1 in a million worldwide (https://ghr.nlm.nih.gov/condition/glanzmann-thrombasthenia#statistics), but in reality, there are no reliable estimates of the worldwide incidence.

Most GT families have private mutations, although a few mutations re-occur in non-related families suggesting mutational hotspots. The incidence is often reported as higher in certain ethnic groups (Iraqi Jews, Palestinian Arabs and French gypsies of the Manouche tribe) where consanguinity is more likely.

GT molecular defects:

Almost all reported causative variants have been in either *ITGA2B* or *ITGB3.* The *ITGA2B* gene is 17 kb and has 30 exons; the *ITGB3* gene is 59 kb and has 15 exons

- Missense variants (most common cause of GT) in any part of gene; retention and degradation in ER/Golgi; some associated with altered exon splicing
- Nonsense variants (premature stop codons)
- Small indels that alter frame and introduce premature stop codons

---

- mRNA splicing defects
- Large deletions, inversions and duplications are rare
- Other rare mechanisms
- Generally, mutations in *ITGA2B* and *ITGB3* produce indistinguishable phenotypes
- Variant GTs are more likely to be caused by genetic changes in *ITGB3*
- Variants in *ITGB3* extracellular Cys residues can cause gain-of-function changes
- A GT-like phenotype has been described in several patients with mutations in *FERMT3* (kindlin-3) and *RASGRP2* (CalDAG-GEFI), genes regulating integrin activation

ACMG Classification Rule Specifications for Glanzmann thrombasthenia (GT)

SUMMARY OF CLASSIFICATION CRITERIA

(Rules in black required no specification. Rules in grey are not applicable to GT. GT specified rules are in red)

PATHOGENIC

Very Strong

PVS1 – Null variant in gene with established LOF as disease mechanism

Strong

PS1 - Same amino acid change from different DNA change

PS2 – *De novo* with paternity and maternity confirmed

PS3 – Well-established in vitro or in vivo, functional studies supportive of a damaging effect

PS4 - Prevalence of variant in affected individuals is significantly increased over controls.

Moderate

PM1 – Present in functional region without benign variation

PM2 – Rarity/Absence in control populations

PM3 - Detected *in trans* with a pathogenic or likely pathogenic variant

PM4 – Protein length changes

PM5 – Missense change at same codon as another pathogenic missense variant

PM6 – Assumed *de novo* with specifications as recommended by SVI WG

Supporting

PP1 – Cosegregation with disease in multiple affected family members

PP2 - Missense variant in a gene with low rate of benign missense variation

PP3 –Multiple lines of computational evidence supporting a deleterious effect

PP4 – Phenotype and family history specific for disease with single genetic etiology

PP5 - Reputable source reports as pathogenic

BENIGN

Stand-Alone

BA1 – Allele frequency cutoff ≥0.24% (99.99% CI w/subpopulation w/min of 5 alleles)

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

Strong

BS1 – Allele frequency cutoff 0.158% (99.99% CI w/subpopulation w/min of 5 alleles)

BS2 - Observed in homozygous state in healthy individuals

BS3 – Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing

BS4 - Non-segregation in affected relatives

Supporting

BP1 - Missense variant in gene where only LOF causes disease

BP2 – Bi-allelic variants for a fully penetrant disorder

BP3 - In-frame deletions/insertions in a repetitive region without a known function

BP4 - Multiple lines of computational evidence support no impact

BP5 - Variant found in a case with an alternate molecular basis for disease

BP6 - Reputable source reports as benign

BP7 - Synonymous variant for which splicing prediction algorithms predict no impact

---

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

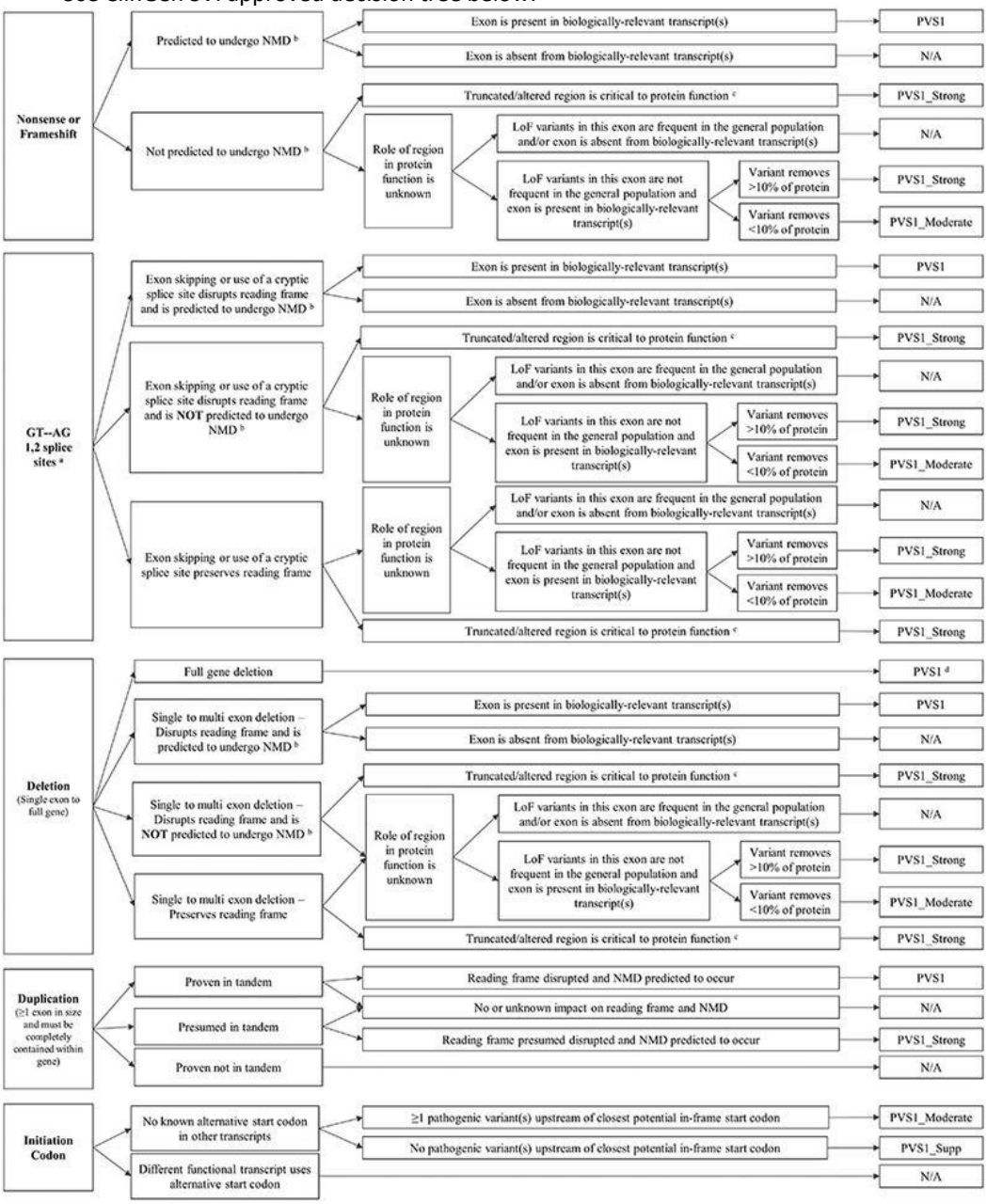
This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

VERY STRONG EVIDENCE OF PATHOGENICITY

PVS1 **Null variant in a gene where LOF is a known mechanism of disease. Null variants to include truncating variants, canonical splice sites, exon gross deletions, intragenic exon tandem duplications, and the initiation codon**

- Use ClinGen SVI approved decision tree below:



Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

- In-frame exons: *ITGA2B* = 7, 8, 10, 12, 13, 15, 18, 21, 23, 25-30; *ITGB3* = 6-9, 13, 15
- Regions "critical to protein function" have been specified to include:
  - Extracellular domains involved in ligand binding:
    - ITGB3 β1 domain, including amino acids 135-197 & 237-240
    - ITGA2B β Propeller domain (amino acids 32-482)
  - The transmembrane domains of ITGA2B (amino acids 994-1019) and ITGB3 (amino acids (719-741)
  - The cytoplasmic domain of ITGB3 (amino acids 742-788)

STRONG EVIDENCE OF PATHOGENICITY

PS1 **Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.**

Example: Val->Leu caused by either G>C or G>T in the same codon.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

PS2 ***De novo*** **(both maternity and paternity confirmed) in a patient with the disease and no family history.**

- Use only when proband has an additional pathogenic or likely pathogenic variant with the *de novo* variant. Additionally, the P/LP variant must have been classified as such by the Platelet VCEP.

- Use ClinGen SVI's proposed point recommendation table to determine the appropriate evidence code weight (see below). To be scored at the highest level with "Phenotype highly specific for gene" the proband must meet the PP4 criteria.

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

Table 1. Points awarded per de novo occurrence

Phenotypic consistency	Points per Proband	
	Confirmed de novo	Assumed de novo
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5
Phenotype consistent with gene but not highly specific and high genetic heterogeneity*	0.5	0.25
Phenotype not consistent with gene	0	0

*Maximum allowable value of 1 may contribute to overall score

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for de novo occurrence(s)

Supporting (PS2_Supporting or PM6_Supporting)	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4

PS3 Well-established in vitro or in vivo, functional studies supportive of a damaging effect on the gene or gene product

- PS3: In a transgenic animal model, must demonstrate minimal to no function (flow cytometry JON/A, aggregation, or alternate assay analyzing integrin function)

-OR-

In a model organism or heterologous cell line, EITHER (A) when expression is normal or reduced (>5% expression compared to WT), disruption of protein function must be demonstrated by significantly reduced binding to fibrinogen or ligand mimetic antibodies (e.g. PAC-1) OR (B) Absent surface protein expression (<5% expression compared to WT) shown by at least 1 of 2 of the following assays: flow cytometry or Western blotting.

- PS3_Moderate: In a model organism or heterologous cell line, significantly reduced surface protein expression (5-25% expression compared to WT) on at least 1 of 2 of the following assays: flow cytometry or Western blotting.

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.

- This rule does not apply to GT due to the rarity of this disorder and lack of appropriate studies. Additionally, most pathogenic variants are private (Nurden et al. Hum Mutat 2015; PMID: 25728920).

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

MODERATE EVIDENCE OF PATHOGENICITY

PM1 **Located in a mutational critical and well-established functional domain/splice site without benign variation**

- This rule does not apply to GT due to the fact that these genes are thought to be highly polymorphic (PMID: 25827233); and there are no known hot spots.

PM2_Supporting **Absent or rare in population databases (gnomAD: http://gnomad.broadinstitute.org).**

- This evidence code is available when a variant is present in fewer than 1 in 10,000 alleles in the ExAC or gnomAD population cohorts.
- This cutoff was recommended based on work from Buitrago, et al showing that none of the established GT pathogenic variants were identified in ~32,000 alleles studies; suggesting that pathogenic variants have allele frequencies less than 0.01% in studied populations (PMID: 25827233)

PM3 **Variant detected *in trans* with a pathogenic variant**

- Both variants must be classified using *ITGA2B/ITGB3* rule specifications.
- Use ClinGen SVI's proposed point recommendation table to determine the appropriate evidence code weight:

Table 1. Points awarded per in *trans* occurrence

Classification/Zygosity of other variant[1]	Points per Proband	
	Confirmed in *trans*	Phase unknown
Pathogenic or Likely pathogenic variant	1.0	0.5 (P) 0.25 (LP)
Homozygous occurrence *(max point 1.0)*	0.5	N/A
Uncertain significance variant on other allele *(max point 0.5)*	0.25	0.0

[1]All variants should be sufficiently rare (meet PM2 specification)

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for PM3

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

PM4 **Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants**

- This rule can be applied as originally intended by the ACMG/AMP guidelines.

PM5 **Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before**

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

- The strength of this rule may be adjusted based on the classification of the previously observed variant.
- PM5: use as written
- PM5_Supporting: Novel missense change at an amino acid residue where a different missense change determined to be *likely pathogenic* has been seen before

PM6 Assumed *de novo*, but without confirmation of paternity and maternity.

- Use only when proband has a pathogenic or likely pathogenic variant *in trans* with the *de novo* variant.
- Use ClinGen SVI's proposed point recommendation table to determine the appropriate evidence code weight (see PS2). To be scored at the highest level with "Phenotype highly specific for gene" the patient must meet the PP4 criteria.

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease (evidence code dependent on number of meiosis and families reported)

- At each strength level the affected relative(s) should have both variants identified in the proband.

Supporting: Segregation in proband plus 1 affected relative when phase is confirmed *in trans*

Moderate: Segregations in proband plus 2 affected relatives when phase is confirmed *in trans*

Strong: Segregations in proband plus >2 affected relatives when phase is confirmed *in trans*

PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

- This rule does not apply to GT due to the fact that these genes are thought to be highly polymorphic (PMID: 25827233)

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product.

For missense variants:

REVEL score of ≥ 0.7 is required for use of this rule code.

Buitrago et al. (PNAS 2015) reported that three commonly used computational algorithms (Polyphen, SIFT, and CADD) to predict Pathogenicity had a 69–98% sensitivity in detecting GT pathogenic variants. (PMID: 25827233).

For splicing variants:

≥2 independent *in silico* missense predictors predict a damaging impact

We have used Human Splicing Finder and Maximum Entropy Scan with the following parameters: (1) the threshold for a position to be considered a splice site is >65 for HSF and >3 for MaxEntScan, (2) a broken splice site is defined as a position

that has shifted below the threshold with a difference in score of <-10% for HSF or <-30% for MaxEntScan, and (3) a new splice site is defined as a position that has shifted above the threshold where the difference in score is >10% for HSF or >30% for MaxEntScan.

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

- Proband must meet diagnostic criteria for GT, which includes phenotypic and laboratory findings. The PP4 code is not used at the original strength. A Bayesian analysis was completed to demonstrate that this phenotype is highly specific and should be counted as a strong level of evidence. Individuals lacking expression data further confirming a diagnosis of GT would be downgraded to a moderate level of evidence.

	Disease				
Test	Present	n	Absent	n	Total
Positive	True Positive	a= 11	False Positive	c= 4	a + c = 15
Negative	False Negative	b= 5	True Negative	d= 525	b + d = 530
Total		a + b = 16		c + d = 529	

Results

Statistic	Value	95% CI
Sensitivity	68.75%	41.34% to 88.98%
Specificity	99.24%	98.08% to 99.79%
Positive Likelihood Ratio	90.92	32.44 to 254.85
Negative Likelihood Ratio	0.31	0.15 to 0.65
Disease prevalence (*)	2.94%	1.69% to 4.72%
Positive Predictive Value (*)	73.33%	49.52% to 88.52%
Negative Predictive Value (*)	99.06%	98.07% to 99.54%
Accuracy (*)	98.35%	96.89% to 99.24%

- PP4_Strong Bleeding phenotype: mucocutaneous bleeding including epistaxis, petichae, easy bruising, oral bleeding, gastrointestinal bleeding and menorrhagia **(proband must have at least one bleeding symptom)**
- PP4_Strong Laboratory phenotype:

  - Full sequencing of the *ITGA2B* and *ITGB3* genes is required to use this code at a strong weight.

  - Abnormal lab values/patterns:

    (1) must demonstrate minimal to no aggregation with all, at least 2, tested agonists (except ristocetin, which must be normal)

    -AND-

    (2) EITHER (A) reduced (≤25%) or absent surface protein expression (demonstrated by flow cytometry or

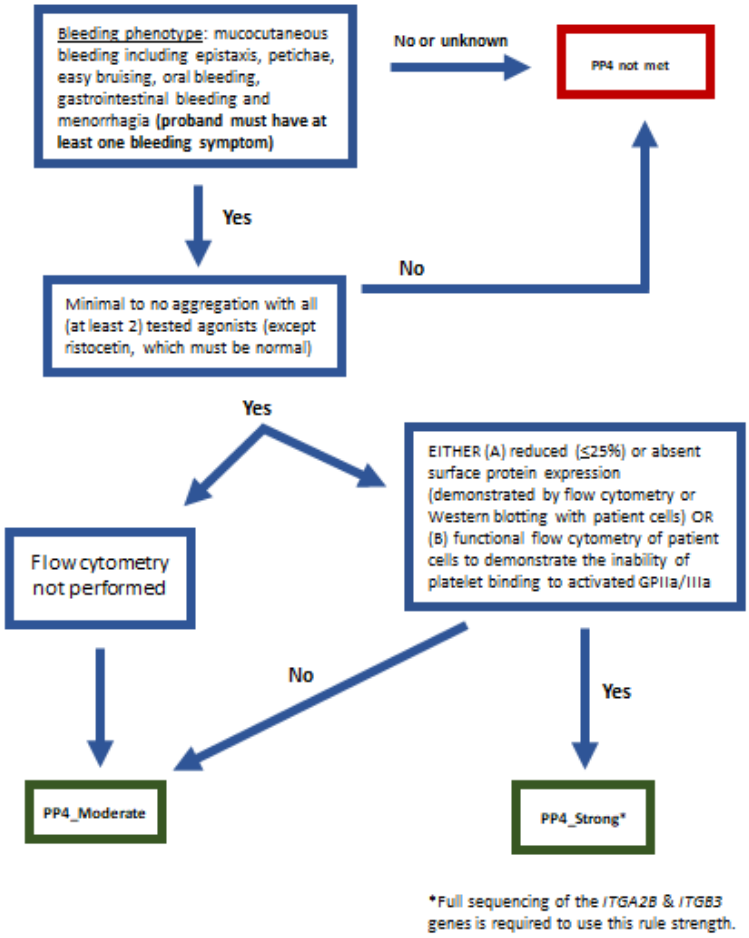
Western blotting with patient cells) OR (B) functional flow cytometry of patient cells to demonstrate the inability of platelet binding to activated GPIIa/IIIa.

- PP4_Moderate Laboratory phenotype:

  - Abnormal lab values/patterns:

  (1) must demonstrate minimal to no aggregation with all, at least 2, tested agonists

  (except ristocetin, which must be normal)



PP5 **Reputable source reports as pathogenic**

- Do not use this rule code

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

STAND-ALONE EVIDENCE OF BENIGN IMPACT

BA1 Allele frequency is greater than expected for disorder

- Use a minor allele frequency cutoff of ≥0.0024 or 0.24% (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

The prevalence of GT worldwide is not well defined. Therefore, we took the conservative approach of using the reported prevalence of 1 in 200,000 in the French Manouche population, which is the highest reported prevalence for this calculation and assumed 100% penetrance. Additionally, the calculator was set to 1 for gene and allele heterogeneity to produce a conservative number.

STRONG EVIDENCE OF BENIGN IMPACT

BS1 Allele frequency is greater than expected for disorder

- Use a minor allele frequency cutoff of >0.00158 but <0.0024 (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

To set the strong MAF cutoff, we use the rationale discussed above and accounted for the two known genes associated with GT; whereas the above calculation assumed only one causative gene.

BS2 Observed in homozygous state in healthy individuals

- This evidence code is available when the variant is identified in ≥1 homozygotes who are unaffected (proven with at least aggregometry).

- It is important to note that in order to use this rule one must have phenotypic information about an individual who is homozygous for a particular variant. This means one could not use population data (i.e. gnomAD or ExAC) as evidence. This code would more likely be used in the setting of gene panel testing, where an individual with a different phenotype was found to be homozygous for a variant in one of those two genes.

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or Splicing

- Must demonstrate normal aggregometry in a transgenic mouse model.

-OR-

- In a heterologous cell line, must demonstrate BOTH normal expression (>75%) and normal protein function, based on binding to fibrinogen or ligand mimetic antibodies (e.g. PAC-1).

BS4 Lack of segregation in affected members of a family

- The variant is not detected in an affected family member, with a confirmed bleeding phenotype and appropriate lab values as described above.

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.

ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

SUPPORTING EVIDENCE OF BENIGN IMPACT

BP1 **Missense variant in a gene for which primarily truncating variants are known to cause disease**

- This rule code does not apply to these genes, as truncating variants account for only a portion of disease causing variants.

BP2 **Observed *in cis* with a pathogenic variant**

- This rule can be applied as originally intended by the ACMG/AMP guidelines.

BP3 **In-frame deletions/insertions in a repetitive region without a known function**

- This rule can be applied as originally intended by the ACMG/AMP guidelines in the *ITG3B* gene, which has three repetitive microsatellite regions. There are no known repetitive regions in the *ITGA2B* gene.

BP4 **Multiple lines of computational evidence suggest no impact on gene/gene product**

- Use this code for missense variants with a REVEL score of < 0.25.
    - This recommendation was based on evaluating the REVEL scores of variants assessed as benign by this VCEP using our rule specifications with the exception of this rule code. All benign and likely benign variants fell under this threshold.
    - We are not recommending the use of other missense *in silico* model predictors at this time due to a previous concern about their validity for these genes as demonstrated by Buitrago et al. (PNAS 2015). They found that the three computational algorithms they used were less reliable predicting non-pathogenicity of previously known benign variants. (PMID: 25827233)

BP5 **Variant found in a case with an alternate molecular basis for disease**

- This rule code is not recommended for use at this time.

- Diminished platelet expression and function of αIIB β3 integrin is very specific for GT. Therefore, pathogenic variants in other genes (such as *FERMT2* and *RASGRP2*) are subtypes of GT in which there is only diminished function of αIIB β3 integrin may have similar laboratory features to other platelet disorders. In these cases, pathogenic variants in other platelet genes may be causative.

BP6 **Reputable source reports as benign**

- Do not use this rule code

BP7 **A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.**

- This rule can be applied as originally intended by the ACMG/AMP guidelines.

Related publication(s): **Date Approved:** September 4, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria for the most recent version.